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A PRELIMINARY NOTE ON THE RELATION OF NORMAL LIVING CELLS TO THE EXISTING THEORIES OF THE HISTOGENESIS OF CONNECTIVE TISSUE.

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The connective issue of the adult is in the embryo derived from the mesenchyme. The same is true of certain other tissues, endothelium, muscle, and cartilage, bone and dentine, if these last be not included within the scope of the term connective tissue. It is in this narrower sense that the term connective tissue is herein used.

In considering the origin of connective tissue one has to take into account two distinct elements, cells and fibers. Of the cells there are at various stages in the embryonic mesenchyme three distinct types: (*a*) the wandering cells, viz., leucocytes, which are more directly concerned with the processes of hæmatopoiesis; (*b*) those cells which give rise to the true wandering connective tissue cells of the mature tissues, the mast cells, plasma cells, etc., whose history is more or less closely concerned with that of the embryonic leucocytes; (*c*) those typical connective tissue cells which are related to the fibers and which are commonly known as the "fixed" connective tissue cells. It is without doubt only the last type which is concerned with the origin of the connective tissue fibers, hence the present note deals only with this type of cell.

At least two types of fibers have also to be considered as arising in the connective tissue mesenchyme, the elastic and the collagenous. We are here concerned only with the latter type, for the reason that the elastic fibers arise at a later period; thus the origin of the connective tissue fibers concerns primarily the collagenous, or so-called "white fibers." It is unnecessary to distinguish at the early stage under consideration between such varieties of collagenous fibers as "reticulum" and "fibrog-

lia," for the former undoubtedly arises in exactly the same manner as the ordinary "white fibers," and of the histogenesis of the latter little or nothing is known if, indeed, it can be considered as an entity distinct from the other connective tissue fibers.

The numerous theories which have been proposed to account for the origin of the connective tissue fibers may be reduced to three chief divisions:

1. The embryonic connective tissue or mesenchymal cells become directly transformed by elongation into connective tissue fibers.

2. The fibers arise in the ground substance between the cells either by transformation of that ground substance or as a secretion from the adjacent cells.

3. The fibers arise within the cells either as distinct fibers (Schwann, 1839), as granules which fuse to form fibers (Spuler, 1896; Lavini, 1909), as an epicellular protoplasmic fibrous layer (Lwoff, 1889), or as an ectoplasm about the cell (Hansen, 1899) or forming the syncytium (Mall, 1902).

The theory of direct transformation by elongation of cells into fibers may well be abandoned and, except it be construed along the lines of the various theories of intracellular origin, it is worthy only of passing notice. Among other things the existence of an overwhelming number of fibers relative to the number of cells present in connective tissue argues against direct transformation, and modern microscopical methods are not able to detect the phases of such transformation either in normal growing tissues or in the process of wound repair. For several decades the results of observations have been in support of either the intercellular or the intracellular group of theories.

To Henle (1841) we owe the theory of the intercellular (extracellular) origin of connective tissue fibers, the fibers being supposed to arise from the intercellular ground substance, not directly from the cells. Latterly this theory has received but little support unless it be from the observations of Merkel (1895), who found that in the umbilical cord fibers appear first to arise in locations relatively remote from the cells. Later the cells wandered into these fibrous areas, and at a still later

period the fibers had so increased in numbers that they necessarily laid in relation to the cells.

The weak point in Merkel's deduction appears to be the disregard of the locomotive properties of mesenchymal or young connective tissue cells. If these cells, except for the incidents of mitosis, are stationary, then Merkel's observations would render conclusive evidence to support the theory of the extra-cellular origin of fibers. But that such cells possess at least a limited power of motion simulating the amœboid character has long been known. An adequate theory must account for the activity of these cells. Merkel's theory presupposes them to be stationary till fibers have appeared and only later to acquire amœboid characters.

In searching for a tissue which would offer opportunity for the study of the activities of connective tissue cells in the living

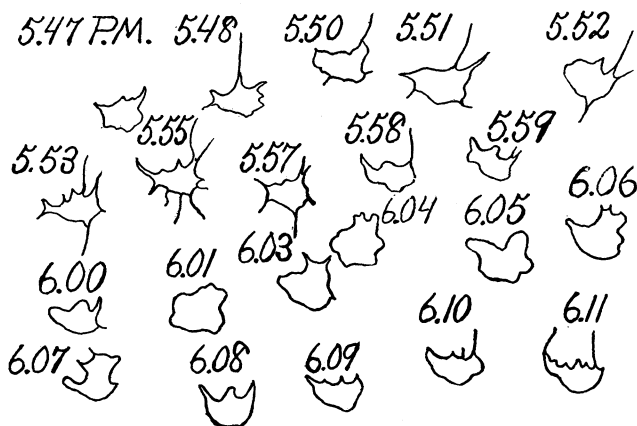


FIG. 1. Outlines of a stellate connective tissue cell in the caudal fin of a *Fundulus* embryo 4.5 mm. long (one day after hatching). The figures record the moment of completion of each drawing, the whole observation extending over a period of 24 minutes. The fish was afterward returned to water and swam for two days before being killed for further histological use. The cell observed corresponds in appearance with that shown by W. Flemming (*Arch. f. Anat.*, 1897, Fig. 9, Plate VI.) in the mesentery of a *Salamander* larva. It shows active amœboid motion and extensive changes of form. Its locomotion was not recorded, it was quite limited. $\times 800$, camera lucida.

animal under normal conditions, I have found that the median fin of young fish embryos affords a subject for study which throws some light on Merkel's observations.

In a *Fundulus* embryo of 6 mm. total length—several days after hatching—I find in the median fin between the double layer of cutaneous epithelium the first signs of the dermal fin-rays. Blood-vessels have not entered, and between the fin-rays are few if any connective tissue fibers, certainly none in the distal portion of the fin. Invading the base of the fin is a mass of mesenchymal cells, mostly of the round cell type. Scattered through the fin are, here and there, isolated connective tissue cells in very limited numbers, distributed over an area representing the proximal one half or two thirds of the fin, and forming a sort of skirmish line, as it were, in advance of the army of round cells. These stellate cells can often be distinctly seen in the living animal. Later, connective tissue cells in abundance wander into the fin and fibers appear. But it cannot be said that the fibers appear in advance of the cells, for the “skirmish line” precedes the appearance of fibers, these advance cells becoming later intermingled with those of the subsequent invasion.

The advance cells, like all other stellate connective tissue cells in the fins of embryo fishes, I find to be actively amoeboid, capable not only of motion but to some extent of locomotion. Since these cells are travelling through an area in which fibers are only just beginning to appear, the location of early fibers at points relatively distant from the cell in “fixed” tissue at once loses its significance and thus robs the theory of the extracellular origin of fibers of one of its strongest supports. My observations may be readily verified on any living, free-swimming *Fundulus* embryo within the first week after hatching.

Connective tissue fibers may be supposed to arise as a product of secretion of the mesenchymal cells, the extracellular deposition of fibers thus occurring under the influence of the cells. This is but a slight deviation from the strict extracellular origin presupposed by the theory of Henle and Kölliker. Such secretion must appear either in fluid form or as granules. In either case it is difficult to conceive why the secretion should take on a linear form if the cells remain stationary, and still more difficult if the cells are in motion, unless that motion be extremely slow and in one direction only.

By observation of the fins of embryo fish, chiefly *Fundulus*, I have been able to observe that the spindle cells of embryonic

connective tissue possess locomotion and move largely in one direction, but their motion and locomotion is extremely active, and so far as I can observe in the living animal they leave behind no trail of secretion, certainly no observable granules: still more important, the fibers appear to have been formed in great numbers prior to the appearance of the type of spindle cells in any considerable proportion relative to the other types of cells, round and stellate, already present in the primitive connective tissue. Hence I cannot conceive of the spindle cells as producing fibers by direct secretion.

But it is at the time of the predominance of stellate cells that the fibers make their first appearance in the fish's fin, as is likewise the case in the tissues studied by other observers. These stellate cells rapidly change their form, throwing out and retracting their processes in rapid succession, as can be seen in any living, free-swimming *Fundulus* embryo under 25 mm. total length. Fig. 1 shows the outline of such an early connective tissue cell in the caudal fin, its changes of form at intervals for a period of twenty-four minutes being accurately depicted with the aid of the camera lucida. It is impossible to both watch and draw all the changes in form occurring during this period, but sufficient are shown to demonstrate very active amoeboid motion. By observing these cells in relation to a relatively fixed object, *e. g.*, a chromatophore, or a joint in an adjacent fin-ray, I have been able to detect in them considerable locomotion. I find that, unlike the spindle cells, the stellate cells appear to travel equally well in all directions. Neither following their locomotion nor in trail of a retracted process do I find any indication of secreted granules, nor of anything other than the most delicate, preformed fibers. One would suspect that if new fibers were being formed by a process of secretion they would appear within twenty-four minutes after such a cell or its process had occupied a given position in which fiber should reasonably be expected to be formed. Though I have repeated the experiment many times I have failed to observe such evidence of secretion, and these results do not appear to be consistent with a theory of direct secretion of fibers by the primitive connective tissue cells.

The intracellular theories of the origin of connective tissue fibers may be divided into two classes, those in which the fibers

are presumed to arise in the peripheral portion of the cell, as proposed by Lwoff (1889), or in a modified peripheral portion of the cellular or syncytial protoplasm, ectoplasm or exoplasm, as proposed by Hansen (1899) and afterwards by Mall (1902), and those in which the fibers are presumed to have a direct intracellular origin as observed by Spuler (1896) and later by Livini (1909).

The "circumcellular" origin of fibers proposed by Lwoff, since it must take into account the separation of fibers from the cells as well as their origin from intracellular granules, does not fundamentally differ from the later ectoplasmic modifications

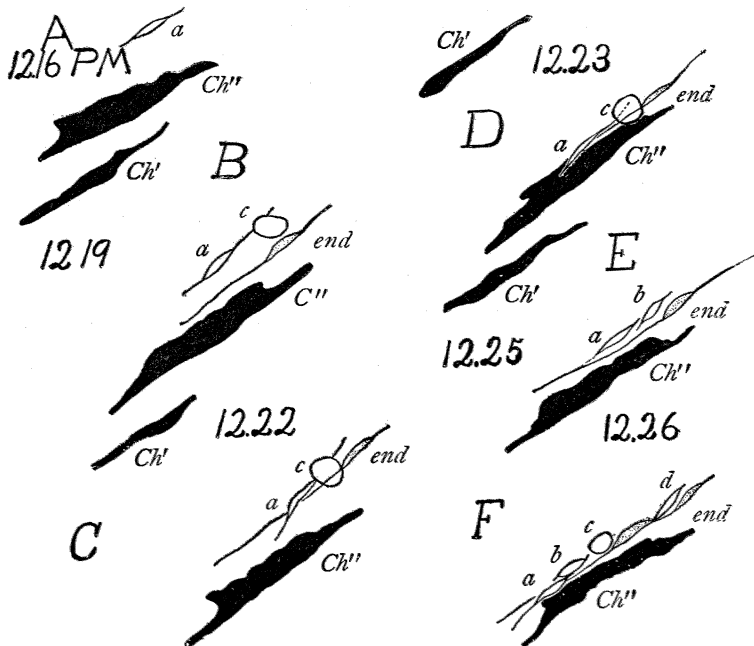


FIG. 2. A record of the locomotion of a spindle connective tissue cell, *a*, for a period of 10 minutes, adjacent chromatophores bordering on the fin-rays on either side being taken as the relatively fixed points. The cell was found to have covered a distance of about 50μ , a rate of 1μ in every 12 seconds. *a-d*, connective tissue cells; *c*, a round cell, the others spindle shaped; *Ch'*, *Ch''*, two chromatophores; *end*, endothelium of an afferent blood-vessel.

of Hansen and Mall. These later authors, basing their investigation on "fixed" material, presuppose a constant but gradual recession of the endoplasmic area with coincident changes in

the exoplasm in order to account for the transference of fibers from a position within the cell, in the ectoplasm, to an extracellular position. This hypothesis is entirely harmonious with the appearances to be observed in "fixed" tissue with the methods used. However, it is open to two objections, viz., the observations of Spuler, confirmed in a casual and not wholly satisfactory way by Livini, that fibers actually lie within the cells, and not always at the periphery, as Hansen's and Mall's theories presuppose, but even in direct contact with the nucleus; and, secondly, if the cells are in active amœboid motion, with locomotion as well, as is certainly the case in the fins of the embryo *Fundulus*, then one can hardly conceive that the more or less fixed syncytium described by Mall, whose observations can be readily verified by any of the usual histological methods applied to the tissue used, is sufficiently stationary to permit the retraction or shrinkage of endoplasm, thus leaving the endoplasmic fibrils outside the bounds of the cellular protoplasm, for according to Mall's theory true cells are lost, being replaced by nucleated endoplasmic areas anastomosing with their neighbors to form a continuous syncytial net.

I have many times observed the fins of *Fundulus* embryos between the lengths of 4.5 mm. (at the time of hatching) and 25 mm., the pectoral and caudal fins being usually selected, and in no single instance have I observed either a stellate or a spindle cell which did not exhibit changes of form and locomotion, the latter being sometimes very limited, at other times, as shown in Fig. 2, quite extensive. Hence the ultimate theory of connective tissue histogenesis, it would seem, must take into account the relative activity of the mesodermic cells. So far as I appreciate them, none of the theories thus far advanced fully meet all of the observed conditions. Further studies of both living and "fixed" tissue may further elucidate the true conditions involved in the histogenic process.

The fins of living fish embryos offer for the development of connective tissue a most desirable subject for study, for in the subcutaneous tissue between the dermal fin-rays one finds a definite connective tissue area, in which, with the exception of the somewhat related chromatophores, there is no other structure

to confuse the picture. This area is bordered on either side by the dermal fin-ray, beneath which an efferent blood-vessel descends, and alongside of which the return or afferent vessel ascends the fin; it is covered only by the pavement cells of the dermal epithelium, whose outlines are sharply defined, lying at a more superficial focal plane than the connective tissue. The chromatophores are easily recognized both by their color, black to yellow, and by their peculiar branching form, so that even those relatively or almost entirely devoid of color can be easily differentiated from the smaller colorless connective tissue cells. Moreover, the chromatophores are prone to lie in immediate relation to the fin-rays and to the blood-vessels.

In conclusion I desire to emphasize the opinion that in the study of histogenesis of connective tissue we must take into consideration the conditions surrounding the living cells and draw conclusions from "fixed" tissue only in the light of our knowledge of the living.

To furnish conclusive results living cells must be observed under normal conditions and not merely under those most unusual, or even pathological, conditions which surround the observation of growing tissues either in the mesentery studied by Flemming and others, open to the criticism of the presence of inflammatory changes, or in the tissue cultures by the more recent method of Harrison, Carrel and Burrows, which is surrounded by the necessity for the interpretation of results arising under entirely new surroundings and conditions as yet but little understood. Both in the mesentery and in tissue cultures movements of the connective tissue cells and even locomotion have been observed. I now add to these former results the record of the observation of motion and locomotion in cells under wholly normal conditions, in animals which underwent no operation other than in some instances the administration of chloretone, and which remained alive, active and normal for some time subsequent to the period of observation. For example, the particular fish which furnished the subject for Fig. 1 was returned to sea water and remained actively swimming for two days; he could have been kept much longer.

For the opportunity of pursuing this study I am indebted to the Marine Biological Laboratory at Woods Hole, Mass.